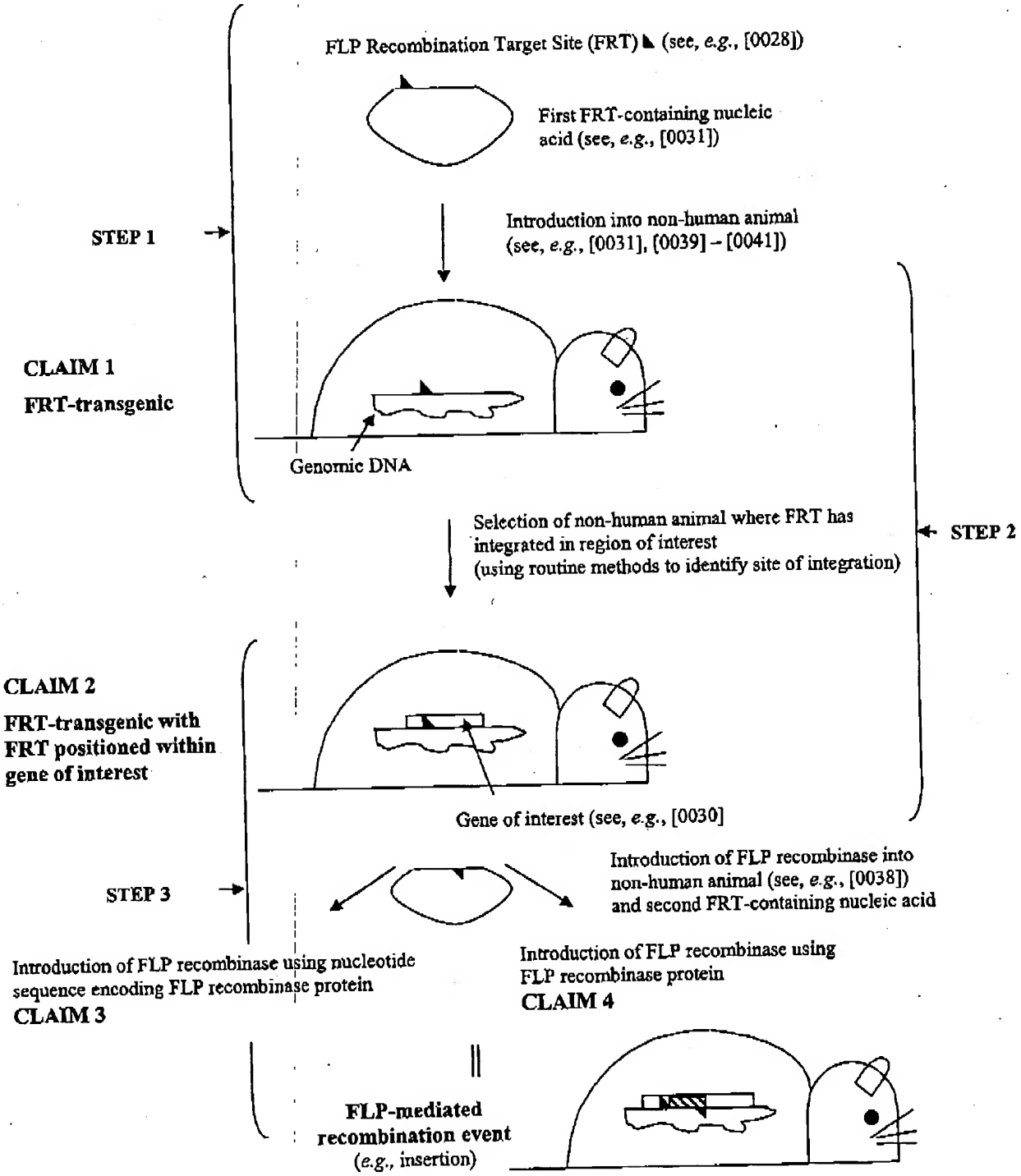
Remarks

Courtesies extended to Applicants' representative at the personal interview held May 18, 2004, are acknowledged with appreciation.

In accordance with the present invention, there are provided transgenic non-human mammals useful as hosts for carrying out FLP-mediated recombination events. A schematic illustration of the present invention is provided below as Figure A.

FIGURE A



Thus, in Figure A, STEP 1, transgenic non-human mammals containing at least one FLP recombination target site in their genomic DNA are prepared by introducing a nucleic acid containing a FLP recombination target site (FRT) into the non-human animal. Following integration of a FRT, as shown in Figure A, STEP 2, a non-human animal may then be selected wherein the FRT has integrated into a region of interest. In another aspect, as shown in Figure A, STEP 3, transgenic mammals containing at least one FLP recombination target site may further contain a FLP recombinase polypeptide or a nucleotide sequence encoding, and capable of expressing, a FLP recombinase, to effect FLP-mediated recombination.

As shown in Figure A, STEP 1, the FLP recombination target site (FRT) is incorporated into the genomic DNA in a first integration event. This integration event can be readily monitored, and the location of the integrated FRT can be readily identified using standard methods known in the art, Figure A, STEP 2. Animals can thus be selected that contain the integrated FRT in a location of interest. Only such pre-selected animals can then be used to target the identified FRT-containing site for a further nucleic acid integration event mediated by FLP recombinase.

Subsequently, as shown in Figure A, STEP 3, the chromosomal integration of a second nucleic acid (*e.g.*, a transgene) is accomplished by FLP-mediated recombination, which controls the site of integration of a second nucleic acid. Invention transgenic animals therefore provide a significant advantage over transgenic animals prepared employing traditional transgenic methodologies (*e.g.*, those that rely on random integration of a transgene into a genome) because the second nucleic acid is selectively integrated at the previously identified and pre-selected target site. In addition, the level, temporal characteristics, and/or tissue distribution of transgene expression in invention transgenic animals may be further regulated. For example, specific promoter systems may be used to control FLP recombinase expression, and thus, to control FLP-mediated recombination of a transgene. Accordingly, the invention provides transgenic animals that can be used in conjunction with a FLP-mediated site-specific integration system to achieve site-specific integration of transgenes, to construct functional genes, to disrupt existing genes, and the like.

Claims 1-19 remain pending in the present application. Claims 12 and 13 have been amended herein to define Applicants' invention with greater particularity. These amendments add no new matter as they are fully supported by the specification and original claims. The present status of all claims in the application and amendments thereto are provided in the listing of claims presented herein beginning on page 2.

The rejection of claims 1-19 under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-13 of U.S. Patent No. 5,677,177 is respectfully traversed. Applicants respectfully submit that the present claims are clearly patentably distinct from claims 1-13 of '177. The present claims are directed to transgenic, non-human mammals; while claims 1-13 of '177 are directed to compositions comprising (i) a FLP recombinase or nucleotide sequence encoding same, and (ii) a DNA comprising a FRT.

Applicants respectfully disagree with the Examiner's assertion that claims 1-13 of U.S. Patent No. 5,677,177 "encompass mammalian cells in vivo, and therefore encompass the transgenic non-human mammals of the instant invention" (see Office Action, at page 3, lines 2-4). To the contrary, claims 1-13 of '177 are directed to compositions which may be used to effect recombination in mammalian cells, and clearly not to the mammalian cells themselves.

Moreover, both the present application and U.S. Patent No. 5,677,177 (Application Serial No. 08/486,409) are divisional applications of the same parent (Application Serial No. 08/147,912), which in turn is a continuation application of Application Serial No. 07/666,252; wherein composition claims and transgenic non-human mammal claims were placed into separate groups pursuant to a restriction requirement. Thus, these groups of claims have already been deemed to be patentably distinct by the USPTO. Accordingly, Applicants respectfully request withdrawal of this rejection of claims 1-19.

The rejection of claims 1-19 under 35 U.S.C. § 112, first paragraph, as allegedly containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to make and/or use the invention, is respectfully traversed. Applicants respectfully submit that one of skill in the art could readily apply standard ES cell technology and transgenic techniques in combination with the novel teachings of the specification to make animals containing FLP target site(s) and to use FLP-mediated recombination in these transgenic animals.

Applicants respectfully disagree with the Examiner's assertion that "insertion of a transgene into [a] randomly located FLP recombination target site, although not random itself, amounts indirectly to random transgene insertion and offers no benefit over direct, random transgene insertion" (see Office Action, at page 5, lines 7-10). Applicants have repeatedly noted that once a FLP recombination target site is integrated into the genome of a mammal (Figure A, STEP 1), its location in a desired position in a mammal is readily identified, for example, using PCR and/or sequencing to identify the position of the FLP recombination target site inserted into the genome (Figure A, STEP 2). After this step, one would then introduce a FLP recombinase protein, or a nucleotide sequence encoding a FLP recombinase (only into such an animal, or cell thereof, where the FRT has been identified as being integrated into a position of interest), to temporally or spatially affect FLP recombinase-mediated recombination (Figure A, STEP 3). Thus, the chromosomal site of transgene integration is controlled, being targeted only in the animal having the previously identified FRT-containing site, and therefore, <u>not random</u> but already identified as a target of interest.

Applicants further respectfully disagree with the Examiner's assertion that "the specification does not provide the guidance necessary to determine how to insert the FLP recombination target site into an active region of the genome" (see Office Action, at page 6, lines 3-4). Applicants have previously noted that the site of FLP recombination target site integration can be readily identified by one of skill in the art, wherever it may be (Figure A, STEP 2). The surrounding sequence can then be readily investigated to determine whether that sequence is a gene of interest, *e.g.*, a known, active region of the genome. There is simply no requirement, *a*

priori, for the FLP recombination target site to be targeted to a known, active region of the genome. From the collection of all sites at which the FLP recombination target site is inserted, one of skill in the art can choose the site of interest. Once a mammal with a FLP recombination target site in a gene of interest is identified, then a FLP recombination event can target an additional nucleic acid fragment specifically to the region of genomic DNA containing that FLP recombination target site.

Similarly, by investigation of the sequence surrounding a FLP recombination target site, one of skill in the art could determine whether that sequence was a region to avoid. For example, if that sequence was known to be associated with a "position effect" that might disrupt subsequent expression of a transgene in that location, that region could simply be avoided by not utilizing such cells or animals for recombination of the transgene. This shows again that the transgene recombination is indeed targeted, and not random, thus providing a significant advantage over standard transgenic techniques.

Once a FLP recombination target site in a gene of interest is identified, Applicants respectfully submit that the introduction of FLP recombinase to affect recombination could readily be performed by one of skill in the art (Figure A, STEP 3). Clearly, protocols for the introduction of nucleic acid encoding FLP recombinase and the optimization of the expression of FLP recombinase in target cells were well-known, as were standard protein expression methods in the art at the time of filing of the present application. Appropriate levels of expression of FLP recombinase can in turn be evaluated by detection of FLP recombination events. Moreover, Applicants respectfully submit that the present claims are not limited to expression of a transgene, but importantly, are also directed to the disruption of a gene of interest using invention transgenic mammals (see, for example, claim 13). Therefore, monitoring FLP recombination events could include assessing expression of the introduced transgene, or reduced (or lack of) expression of the target gene in the genome.

With respect to the species recited in the present claims, the Examiner has acknowledged that "techniques for making non-mouse species of transgenic non-human mammals are standard in the art" (see Office Action, at page 9, lines 6-7). Indeed, such standard techniques can be used to deliver a transgene comprising an FLP recombination target site *in vivo*. The fact that these known techniques result in random transgene insertion is irrelevant as only mammals or cells containing the desired target site are utilized for subsequent insertions mediated by FLP recombinase. Once such standard techniques are utilized to insert a FLP recombination target site into a non-human mammal, and the animal in which the FLP recombination target site is determined, only this animal or cells thereof is used for the second insertion event, i.e., the FLP recombinase-mediated event, provides the asserted advantages of the present invention.

Therefore, one of skill in the art could readily make and use the transgenic mammals as claimed herein by following the teachings of the specification in light of the knowledge in the art at the time of filing. Contrary to the Examiner's assertions, there is neither a requirement of an actual reduction to practice (see Office Action, at page 11, lines 12-13), nor a specific site within the genome for integration of the FLP recombination target site (see Office Action, at page 11, lines 13-14) since the claimed mammals have broad applicability to any region or gene of interest. Accordingly, Applicants respectfully request reconsideration and withdrawal of this rejection of claims 1-19 under 35 U.S.C. § 112, first paragraph.

The rejection of claim 12 under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention, is respectfully traversed. Applicants respectfully submit that claim 12 is clear and definite as previously presented. However, in efforts to advance prosecution and reduce the issues, claims 12 and 13 have both been amended to replace the phrase "said second DNA" with the phrase "said additional DNA fragment". Accordingly, Applicants respectfully request reconsideration and withdrawal of this rejection of claim 12 under 35 U.S.C. § 112, second paragraph.

Conclusion

In view of the above amendments and remarks, prompt and favorable action on all claims is respectfully requested. In the event any matters remain to be resolved in view of this communication, the Examiner is encouraged to call the undersigned so that a prompt disposition of this application can be achieved.

Respectfully submitted,

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